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Chromatin organization and nucleolar vacuoles in *Allium ascalonicum*

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Research Article

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Abstract: The arrangement of chromosomes inside the nucleus is the focal point of the cutting-edge cytogenetic. The chromosomes territories (CT) are studied with numerous technique along with molecular in addition to molecular cytogenetics. The interphase nuclei in the Allium ascalonicum with one to three vacuoles had not been studied so far. In the present study, the interphase nuclei of the material tried to address the causal and role of these structures in the nuclear architecture throughout the cell cycle. The diploid number of chromosomes is 16. The root tip meristematic cells were stained with acetocarnmine (2%), C-banding for heterochromatic regions, and Ag-NOR stain for the nuclear organizer region. The mitotic division of Allium ascalonicum has been studied from meristem cells of root tips by squashed method. The presence of numerous non-dividing cells give the important information on nuclear architectures both on spatial and temporal of these sixteen chromatids. The interphase nuclei in this shallot showed one to three lacunae/vacuoles. Many cells of both dividing and non-dividing nuclei were screened. The finding leads to the conclusions that the vacuole/lacunae are the actually space occupied by nucleolus. It seems most likely that the chromosomes are floating above the nucleolus during the interphase while attaching to the nuclear membrane and reestablished after the anaphase-telophase stage in the succeeding cycle. The lacunae are the spaces leftover by lateral arms of the chromosomes (two lateral vacuoles/lacunae) and telomeres of the sixteen chromosomes. The present studies strongly convinced to speculate the studies on nucleolus of the plant as well as animals will be some part not the whole of the nucleolus. So the outlook on nucleolus should be reevaluated all together for a better understanding of this nuclear organelle. The future line of the research should be using FISH with centromeric and telomeric probes so as to visualize the validity of the present results.

Keywords: Nucleolus, chromosomes, floating, NOR-vacuoles, laterals, telomeric.

INTRODUCTION

Ever since Rabl (1885) in animal and Strasburger (1905) in plant respectively argued for territorial organization of chromosomes in nucleus changed the concept of positions of chromosomes inside the nucleus and finally, Boveri (1909) introduced the term of “Chromosome Territories (CTs)” to confirm the idea of nuclear architectures. There are various techniques developed recently for studying the chromosomes territories. DNA sequences interacting in vivo with DNA binding proteins (van Steensel and Henikoff, 2000), another approach with a great potential for nuclear architecture studies, called ‘‘chromosome conformation capture’’ (3C), was introduced by Dekker et al. (2002). The aggregate of round chromosome conformation capture (4C) with DNA microarrays (Gondor et al., 2008; Schoenfelder et al., 2009) or vastly parallel sequencing (Lieberman-Aiden et al., 2009) has allowed for the primary time mapping of DNA–DNA interactions in cis and trans at a genome-extensive level. But all the above advance molecular techniques have certain disadvantages, one is unable to the visualized whole of the cells/chromosomes in the study.

The squashed technique is an ideal method to study the nuclear architecture: the cells are maintained in their usual position or characteristics features whether interphases or dividing stages despite the absence of the above-sophisticated devices. The only demerit of this technique is the conversion of three-dimensional position into two- dimensional position. This shortcoming could be replenished with the observation of a huge number of cells and speculation of plausible conclusions on the basis of the observations. An attempt is made to visualize the chromosome territories and consequent spatial and temporal positions of the chromosomes inside the nuclear boundaries using the nuclear structure of the meristematic cells of Allium ascalonicum.

While examining the chromosomes of shallot collected there isthe numerous number of interphase nuclei with 1, 2, and 3 (rarely) non-stained roughly circular hollow structures, which are called nuclear lacunae/vacuoles (NC) hereafter. The rationales behind the present study are A) why and how are variable numbers of NCs formed? B) What are the significances of such NCs in the cells? D) Do these structures are also represented nuclear architectures? Lastly, E) is there

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any relationship between the nucleolus and chromosome arrangement? The present paper reports the fact that the nuclear architecture of the chromosomes are inherited from parents to daughters through anaphase –telophase chromatids as reported by maintaining the Rabl arrangement. The structures of vacuoles found in the interphases are predetermined by the anaphase chromatid disjunction. Finally, we found that the number of the NCs in the nucleus is mainly because of the plane of inclination and orientation of the mitotic apparatus.

MATERIALS AND METHODS

Plant material used to study the Mitosis: The bulbs of Allium ascalonicum L. from three different localities namely Mayang Imphal, Khurai, and Heinoubok were collected in the month of September 2015 from the local farmers (fig. 1). Allium ascalonicum L. had been planted within the laboratory in BOD incubator at 280 C on a Petri-plate with water and after 24 hours, root hints of average 0.5 cm had been dealt with with 0.fifty six % KCl for one hour later fixed in fixative (3:1 ethanol and glacial acetic acid with the aid of quantity) for twenty-four hours and preserve in 70% ethanol. a number of root hints had been pretreated with zero.five% Colchicine (Himedia RM342 10g) solution for four hours at room temperature previous to KCl remedy, for the observe of metaphase chromosomes for ascertaining the diploid count.

Slide preparation: In a test tube, 1 ml of Acetocarmine (2%, Merck, India- C. I. No. 75470, S. No. 1381) except for Ag-NOR (no acetoramine, instead water) mixed with 20µl each of 45% glacial acetic acid and 1NHCl were taken along with seven root tips of Allium specimens and were warm for 10 minutes over sprit lamp. Meristematic cells from the soften root tips were used for micro slides preparation by squashed method to obtain the different stages of mitotic cells. The slide cover of distained slides were taken out with ice or with 1:1 Ethanol Glacial acetic acid (without stain Acetocarmine)

Fifty cell plates were used for each stage and photographs of best 5 were selected for each stages starting from Interphase, Prophase, Metaphase, Anaphase and Telophase. Nuclear architecture is studied from 2000 non-dividing cells from non-colchicine treated nuclei.

NOR-staining: Prior to Ag-NO2 staining the slides were treated in 5XSSC at 600C for 15 minutes. Then slides were stained for Ag-NOR following Goodpsture and Bloom (1975) with slight modification. Four drops of Ag-NO2 (70%) and Gelatin formic acid (2% and 1% respectively) were put on each slide and covered with cover slides after avoiding bubbles. The slides were incubated inside a hot water bath steamed at 600C for one hour. The cover slides were then removed and washed with double distilled water, air dried and finally mounted in DPX. After 24 hours the slides were examined under the microscope.

C-Banding: The root tips were heated in the solution of one milliliter each of 2XSSC and Acetocarmine (2%) in a test tube till the root tips were softened for squashed methods. Finally the dividing and non-dividing cell plates were screened for C-banded chromosomes.

All the observations were done in 40X objective lens of the BX 41 Olympus Phase contrast microscope and microphotographs were taken with a digital camera attached on it.

RESULTS

The diploid chromosomes of Allium ascalonicum were 16 with karyotypic formula of 2 M (Metacentric chromosomes) + 12 SM (Submetacentrics) + 2ST (Subtelocentric) chromosomes (fig. 2) in all the metaphases from the three habitats.

The overall mitotic division in Allium ascalonicum through the Acetocarmine-squashed method was quite similar as other materials including red and brown onion reported. In the early prophase, the duplicated chromosomes were arranged as in fig. 3 D in which the chromosomes were elongated curved leaving two vacuoles (non-acetocarmine stained for the time). It seems that the centromeres and telomeres were in two different domains when seen in condensed chromosomes (fig. 3 E and F) as reported as Rabl arrangement. The early metaphase in which the duplicated having the two sister chromatids were somewhat concentrated around the certain plane and condensed and aligned at the equatorial plate (fig. 3 G and H). The sister chromatids are separate and ready for the destined journey (fig. 3 I). The sister chromatids were pull towards each poles during anaphase (fig. 3 J-N). During the journey, the p arms were outside and q arms occupied the interior of the circular arrangement or rosette arrangement. After the maximum condensation the chromatids, the decondensation started and the rosette arrangement seemed to be collapsed and chromatids became repelled and formed two lacunae possible to observe at these moments during telophase (fig. 3 O-U). The lacunae persist after cytokinesis. The lacunae were much prominent during G1 and G2 phase of interphase (fig. 3 U-W). The duplication of the chromosomes were not non-random and started at specific site non-heterochromatic regions in the nucleus (fig. 3 A). After duplication,the some arrangement of the chromosomes could be seen in many cell plates (fig. 3 B and C).

Interphase nuclei: The different chromosomes in different stages of the cell cycle were as seen in fig. 4 A, B, C, D, E, and F. During G -1 and G-2 stage the interphase nuclei with one to three lacunae/vacuoles which are named as lateral vacuole (two numbers of LV) both side of centromeric heterochromatin (CH) and one telomeric vacuole (TV). The three vacuoles could be differentiated as largest telomeric, bigger lateral one which are formed by the longer chromosomes while the smallest one

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formed by the shorter chromosomes.

C-banding: C banded nucleus in the fig. 5 showed heterochromatic regions in prophase, metaphase, anaphase, telophase, G2 and G1 phase. Even though heterochromatic regions are not distinct could be due to lesser content of heterochromatic components or might be due to lesser exposer of 2SSC, but the three lacunae could be clearly seen as the most prominently as non-stain parts particularly in the G1 phase (fig. 5 G).

NOR-stain: Ag-NOR and counterstained with Giemsa (2%) stain showed the nucleolus in the anaphase, telophase, the G2 phase and G1 phase (the nucleolus in white arrow and chromatins in black arrow). The lacunae are filled with the conventional black dots in the G1 phase (fig. 6 G). The other results were that the nucleolus diminished as in cell progressed to the division stage and increased in sized as progressed to interphase.

All the above observations undoubtedly lead to speculate the somatic cell cycle in the Allium ascalonicum as shown in the fig. 7.

DISCUSSION

The heavily Ag-impregnated areas of nucleoli are reported by many studies on Allium species and plants (Moreno et al., 1989; Ploton, Menager and Adnet, 1989; Qin et al., 2010; Liu et al., 1985; Qin et al., 2010; Tao et al., 2001).

The present study is in accordance with Rabl (1885), Santos et al (2015) in which the centromeres and telomeres in two different domains during interphase as well as division. Santos et al (2015) argued that the Rabl arrangement was absent in rice, but in fact they might be seeing the vertical centromeric vacuole (CH in the present study) as nuclei are round, could be any point when observed. The present study reveals that the chromosomes are positioned in such a way that the arms formed two lateral lacunae/vacuoles and the telomeres of the 16 chromatids leading to three vacuoles/lacunae during interphase which are predetermined by anaphase-telophase. The present studies if proven correctly, then the studies on nucleolus of plant as well as animals will be some part not whole of the nucleolus as the chromatin engulfed most parts of the nucleolus leaving only. So the outlook on nucleolus should be reevaluated all together for better understanding of these nuclear organelles. One of the study materials for proper understanding of the nucleolus will be polytene chromosomes or salivary gland chromosomes in which minimum chromatin attaching on the nucleolus.

The formation of nucleolus and orientation of chromatins also involved in nuclear architectures. The centromeres remained clustered together just above the telomeric vacuoles while the arms are spread on nucleolus exposing the two lateral vacuoles. Future works should focused on chromosomes numbers arranged at respective regions.

The conclusions are 1) the interphase nuclei had three vacuoles which are filled with nucleolus leading to speculate that the chromatins float or attached to the nucleolus during the interphase and the same is maintained after anaphase-telophase stage. The number of NORs reported so far are the in fact are the exposed part of the nucleolus and number varies in accordance to the orientation of the division, say if upside down will see only telomeric while oblong will show two while twisted oblong will exposed all three lacunae. Hence more intense works before and after bleaching of the chromatins should be the way for proper understanding of the structures and functions of the NORs in both plant and animal materials.

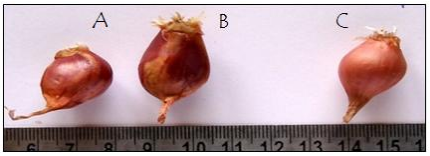

Figure 1. The onion bulbs used in the present study: Heinoubok (A), Khurai (B) and Mayang Imphal (C). Bar represent 1 cm.

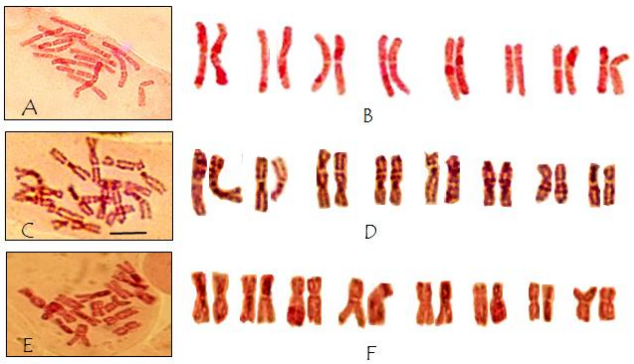

Figure 2. The metaphase plates and respective karyotypes of the onions from different localities from Mayang Imphal (A and B); Khurai (C and D) and Heinoubok (E and F) with identical karyotypic formula of 2M + 12 SM + 2ST chromosomes (Heinoubok). The abbreviations M =Metacentric chromosomes, SM =Submetacentrics, ST = Subtelocentric chromosomes. Bar represents 10 µm.

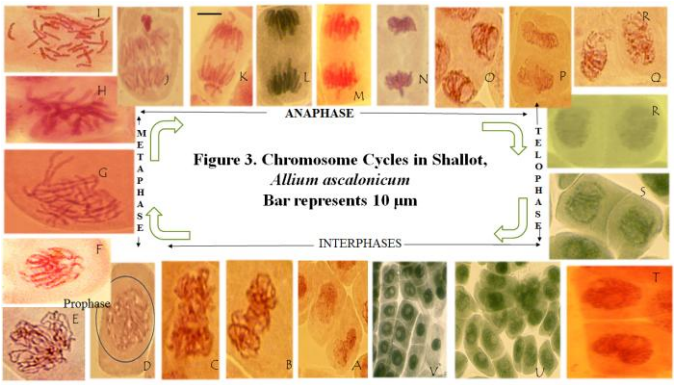

Figure 3. Chromosome Cycles in Shallot, *Allium ascalonicum.* Bar represents 10 µm

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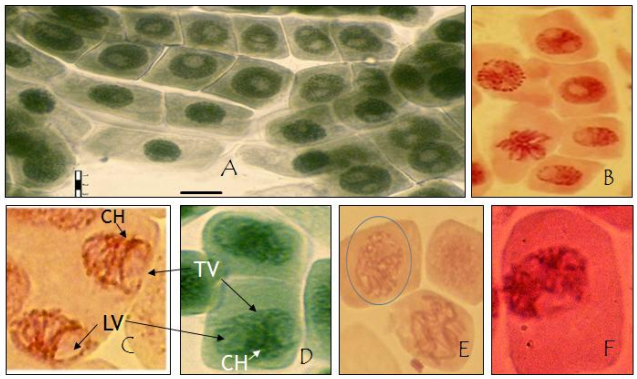

Figure 4. The different chromosomes in different stages of cell cycle. A) the G-2 staged interphase nuclei with one to three lacunae, B) Metaphase plates with interphase nuclei, C) the anaphase plate –up lateral view and down polar view, from the *Allium* where chromosomes are in rosette pattern, the homologous chromatids go side by side; D) late telophase nuclei with distinct lacunae; E) various chromosomes movements inside the nuclei and F) the G-1 nuclei with distinct lacunae. CH = Centromeric heterochromatin, 2X LV = Lateral vacuole, TV = Telomeric vacuole. Bar represents 10 µm.

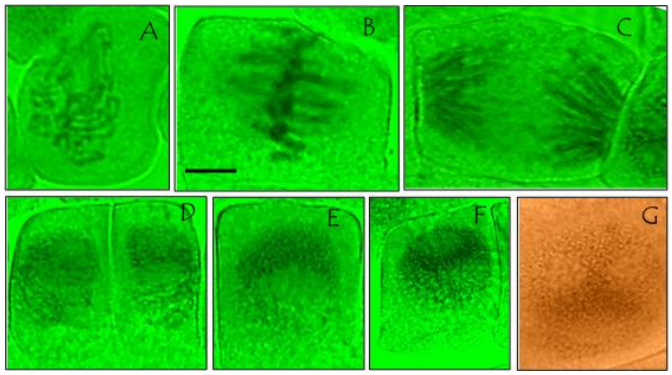

Figure 5. C banded micrographs: A) the prophase, B) metaphase, C) anaphase, D) telophase, E) G_2 in, F) G_2 phase in another view and G) G_1 phase. Bar represents 10 µm.

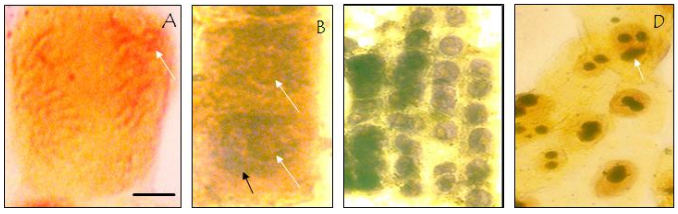

Figure 6. Ag-NOR and counter stained with Giemsa stained micrographs: A) the anaphase, B) telophase, C and D) the G_2 phase and G_1 phase showing the nucleolus in white arrow and chromatins in black arrow. Bar represents 10 µm.

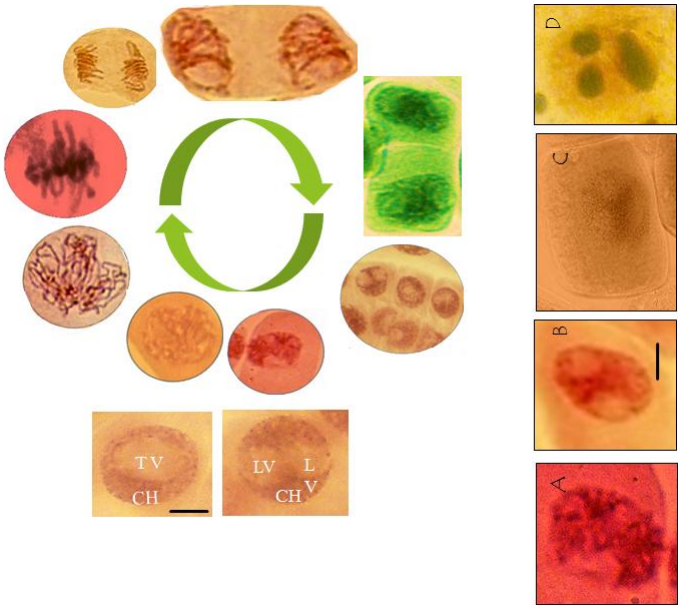

Figure 7. The speculative chromosome cycles in the *Allium ascalonicum*, on the basis of three nuclear lacunae. Bar represents 10 µm.

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